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DRAWINGS ATTACHED

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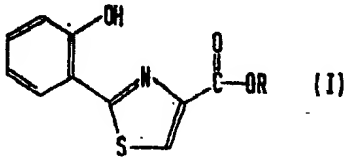
(54) A METHOD OF PREPARING AERUGINOIC ACID AND ESTERS THEREOF

(71) We, GODO SHUSEI KABUSHIKI KAISHA, of No. 2—10, Ginza 6-chome, Chuo-ku, Tokyo, Japan, a Japanese Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a method of preparing aeruginoic acid and esters thereof.

Aeruginoic acid is a compound which was produced and discovered by the inventors by culturing strains of *Pseudomonas aeruginosa* having the ability to assimilate hydrocarbons. The inventors have investigated the chemical structure of aeruginoic acid and have found that it is 2-(*o*-hydroxyphenyl) - thiazole - 4 - carboxylic acid of formula (I) below, where R is hydrogen.



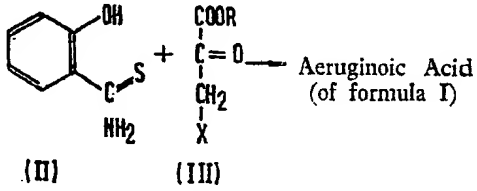
(I)

Aeruginoic acid is described and claimed in our co-pending U.K. Patent Application No. 44235/70 (Serial No. 1,292,169).

Aeruginoic acid has autimicrobial activities against *Botrytis* sinerea and anti-inflammatory activity against *carrageenin adema.*

The methyl ester of aeruginoic acid (of formula (I), where R is a methyl radical) can be isolated from a culture medium on which *Pseudomonas aeruginosa* has been cultured.

The invention provides esters of aeruginoic acid (of formula (I) where R is an organic radical derived by the removal of a hydrogen atom of an alcoholic hydroxyl group from an organic compound and a method for their preparation, comprising reacting *o*-hydroxy - thiobenzamide (of formula (II) below with an ester of halopyruvic acid (of formula (III) below where R is the organic radical and X is a halogen atom). The reaction may be carried out at ambient temperature or at an elevated temperature, conveniently a temperature in the range 60 to 70°C.



(II) (III)

The ester of halopyruvic acid may be an alkyl ester, i.e. an ester of formula (III), where R is an alkyl group. The use of such an ester gives an alkyl ester of aeruginoic acid, i.e. an ester of formula (I), where R is an alkyl group.

The ester of halopyruvic acid and the *o*-hydroxy - thiobenzamide are preferably reacted in equimolar proportions preferably in a reaction medium selected from ethers such as diethyl ether, tetrahydrofuran or dioxane, or an alcohol such as ethanol or methanol or a dipolar solvent such as dimethylformamide.

By the method of the invention it is possible to prepare an ester of aeruginoic acid in high yield in a single step.

An ester of aeruginoic acid can easily be hydrolized to aeruginoic acid and a high yield of aeruginoic acid may be so obtained in an alkaline medium. It has been found that aeruginoic acid thus obtained has the same properties as aeruginoic acid produced by culturing of strains of *Pseudomonas aeruginosa.*

The invention is illustrated by the follow-

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ing Examples. The accompanying drawing shows that aeruginoic acid produced in accordance with the present invention has the same infra-red absorption spectrum as that of aeruginoic acid produced by culturing *Pseudomonas aeruginosa.*

Example 1

Preparation of the ethyl ester aeruginoic acid.

1.4 grams of the ethyl ester of bromo-pyruvic acid were dissolved in 30 mls. of absolute alcohol with agitation and to the solution was added drop by drop one gram of o - hydroxy - thiobenzamide dissolved in 15 mls. of absolute alcohol at room temperature. The resultant mixture was heated at a temperature of 60°C for one hour with agitation to produce a precipitate. The precipitate was filtered and charged into 50 mls. of an aqueous solution containing sodium carbonate and then an ethyl ester of aeruginoic acid was extracted with ethyl acetate. The extract was washed with water and then dried on crystalline sodium sulphate by removing ethyl acetate used as the solvent to produce 1.6 grams of the crystallized ethyl ester of aeruginoic acid.

The product had a melting point of 107°C and its infra-red absorption spectra showed bands at 3120 cm^{-1}, 1725 cm^{-1} and 1220 cm^{-1}. The product was subjected to elemental analysis and the result was compared with the theoretical values calculated as

$C_{12}H_{11}NO_3S$

as listed below.

Analytical values:
C=57.66%, H=4.34%, N=5.62% and S=13.20%

Theoretical values:
C=57.82%, H=4.45%, N=5.62% and S=12.84%

Example 2

Preparation of aeruginoic acid.

0.7 gram of the ethyl ester of aeruginoic acid was mixed with one gram of potassium hydroxide, 3 mls. of water and methanol and the resultant mixture was heated at 60°C for 3 hours and then the mixture was allowed to stand overnight at room temperature. The mixture was poured into water and treated with ether for removing the neutral substance. The ether extract was acidified with diluted hydrochloric acid to produce crystals of aeruginoic acid and then the crystals were extracted with ethyl acetate. The resultant extract was washed with water and then dried on crystalline sodium sulfate and the ethyl acetate was distilled for obtaining 0.5 gram of crude crystals of aeruginoic acid. The crude crystals were washed with methanol and filtered to obtain 0.3 gram of pure crystalline aeruginoic acid.

The product had a melting point or sublimation temperature of 207°C and it decomposed at a temperature of 270°C to 271°C in the same manner as in aeruginoic acid produced by culturing *Pseudomonas aeruginosa.*

The infra-red absorption spectra and the N M R spectra of the product were determined and it was found that the results were the same as those of aeruginoic acid produced by culturing of *pseudomonas aeruginosa.* Still further, the product was subjected to elemental analysis and the result was compared with the theoretical values as listed below.

Analytical values:
C=54.46%, H=3.26%, N=6.26% and S=14.81%

Theoretical values:
C=54.30%, H=3.19%, N=6.33% and S=14.47%

WHAT WE CLAIM IS:—

1. A method of preparing an ester of aeruginoic acid, (2 - (*o* - hydroxy - phenyl)-thiazole - 4 - carboxylic acid, comprising reacting *o* - hydroxy - thiobenzamide with an ester of a halo-pyruvic acid.

2. A method as claimed in claim 1, wherein the reaction is carried out at ambient or elevated temperatures.

3. A method as claimed in either preceding claim, wherein the reaction is carried out at 60 to 70°C.

4. A method as claimed in any preceding claim, in which the *o* - hydroxy - thiobenzamide and the ester of halo pyrubic acid are reacted in equimolar proportions.

5. A method as claimed in claim 4, in which the ester of halo-pyruvic acid is an alkyl ester.

6. A method as claimed in any preceding claim, wherein the reaction is carried out in an ether, an alcohol or a dipolar solvent.

7. A method as claimed in claim 6, wherein the reaction is carried out in diethyl ether, tetrahydrofuran, dioxane, ethanol, methanol or dimethylformamide.

8. A method of preparing an ester of aeruginoic acid, substantially as described herein with reference to Example 1.

9. An ester of aeruginoic acid prepared by a method as claimed in any preceding claim.

10. A method of preparing aeruginoic acid, wherein an ester as claimed in claim 9 is

hydrolysed to aeruginoic acid in an alkaline medium.

11. A method of preparing aeruginoic acid, substantially as described herein with reference to Example 2.

12. Aeruginoic acid prepared by a method as claimed in either of claims 10 and 11.

13. An ester of aeruginoic acid.

MARKS & CLERK.

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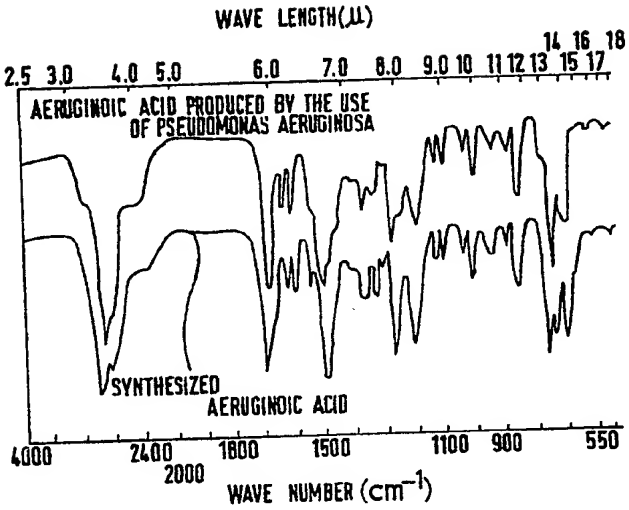
WAVE LENGTH(μ)
2.5 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10 11 12 13 14 15 16 17 18
AERUGINOIC ACID PRODUCED BY THE USE OF PSEUDOMONAS AERUGINOSA
SYNTHESIZED AERUGINOIC ACID
4000 2400 2000 1800 1500 1100 900 550
WAVE NUMBER (cm⁻¹)